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REVIEW ARTICLE

Update on Active Specific Immunotherapy With Melanoma Vaccines

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Although a randomized clinical trial has yet to show a statistically significant improvement in the survival of patients receiving vaccine therapy for malignant melanoma, several studies have shown enhanced survival of patients developing an immune response to a melanoma vaccine. The knowledge and techniques of modern molecular biology and immunology suggest multiple strategies to augment this response. The challenge of immunotherapy research is to determine which combination of approaches leads to a favorable clinical response and how to monitor that response effectively. This review identifies components of a successful vaccine, discusses new ways to modulate and stimulate the immune system, and summarizes some of the more interesting clinical trials of melanoma vaccine immunotherapy. *J. Surg. Oncol. 1997;66:55–64.*

KEY WORDS: **immunomodulation; antigen; humoral/cellular immunity; autologous/allogeneic vaccines**

INTRODUCTION

Some 200 years ago, Dr. Edward Jenner vaccinated a patient against smallpox, using material harvested from pustules on the hands of a milkmaid afflicted with cowpox. His work marked the advent of prophylactic vaccination against potentially fatal infectious illnesses. Approximately 100 years later, active immunotherapy was extended to neoplastic diseases. Light microscopy demonstrated an inflammatory infiltrate in tissues from certain tumors; an apparent correlation between this infiltrate and improved clinical results was reinforced by Dr. William B. Coley's observation of tumor regression in a patient with a systemic bacterial infection. Coley's subsequent use of live and heat-killed *Streptococcus* and *Serratia* organisms (Coley's toxin) to treat patients with various tumors [1] eventually proved unsuccessful, but nonetheless heralded the current era of cancer vaccine research.

Melanoma has become the paradigmatic solid tumor for immunotherapy, partly because it is poorly responsive to conventional systemic treatments [2], but mostly because it is one of the most immunogenic solid tumors. For example, laboratory studies show that blood from melanoma patients contains antibodies against tumor antigens [3,4], as well as cytotoxic T cells (CTL) that can destroy tumor cells in vitro [5,6]. Clinical studies indicate that 3–15% of all cutaneous melanomas are first diagnosed as lymphatic or visceral metastases without evidence of a primary tumor, which suggests that the immune system has caused complete regression of the primary melanoma [7]. Histopathologic evidence of regressive changes has been reported in up to 58% of primary melanoma specimens [8–11]. On rare occasions, there has been spontaneous, complete regression of metastatic disease.

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TABLE I. Nonspecific Immune Enhancers

Intact micro-organisms
Bacille Calmette-Guérin (BCG)
Corynebacterium parvum
Microbial cell wall products
BCG skeleton
Polysaccharides (yeast and fungi)
Glucans
Immunogenic proteins
Keyhole limpet hemocyanin
Haptens
Dinitrophenol
Trinitrophenol
Purified fractions
Lipopolysaccharides
Lipid A
Peptidoglycans
Alkylating agents
Cyclophosphamide
Combinations
DETOX: Lipid A and *Mycobacterium phlei*

TABLE II. Some Immunogenic Antigens Identified in Human Melanoma Cells*

Tumor-associated antigens (TAA)[a]	Melanoma-associated antigens (MAA)[b]
Urinary TAA (glycoprotein 90)	Lipoprotein 180
Fetal antigen (glycoprotein 70)	Tyrosinase
810 peptide (43 kd)	MART-1/Melan A
MAGE 1	Glycoprotein 75 (gp75.TRP)
MAGE 3	Glycoprotein 100 (gp 100/pmel 17)
GM_2	High molecular weight melanoma antigen
GD_2	
O-acetyl GD_3	
GM_3	

*Adapted from Morton and Barth [18], with permission of Lippincott-Raven Publishers.
[a]Found not only in melanoma, but also in kidney, lung, breast, and other solid neoplasms.
[b]Found primarily in melanocytes/melanoma (and rare neoplasms of neural crest origin).

The field of melanoma vaccine immunotherapy has grown too rapidly to be covered in this brief review. Instead, we identify components of the immune system that are key to developing a successful vaccine, discuss new ways to modulate and stimulate the immune system, and summarize some of the more interesting clinical trials of melanoma vaccine immunotherapy.

IMMUNOTHERAPY AND IMMUNE RESPONSE

Immunotherapy is specific or nonspecific. Specific immunotherapeutic agents such as the current melanoma vaccines upregulate the antibody response or the cytotoxic T-cell response to specific tumor antigens. Nonspecific immunotherapeutic agents such as intact microorganisms, microbial cell wall products, haptens, glucans, and protein products stimulate the immune system without targeting specific antigens (Table I). A nonspecific agent is usually administered as an adjuvant before or with a vaccine to enhance the overall immune response and augment the specific response induced by the vaccine. Bacille Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis,* is one of the best-known adjuvants and was an initial focus of melanoma immunotherapy research. Early studies reported encouraging results for BCG alone in melanoma [4,12], but a large prospective trial randomizing patients with high-risk stage I melanoma to two different types of BCG or a control arm failed to demonstrate a survival advantage [13]. At present, no nonspecific immunotherapeutic agent alone has been shown to improve survival, and no adjuvant is clearly superior for use with melanoma vaccines.

The immune response to active immunotherapy is humoral and cell-mediated. Humoral immunity involves antibody production from mature B lymphocytes and usually requires the presence of antigen-specific helper T cells. Cell-mediated immunity (CMI) involves stimulation of CD4+ T cells by an antigen present on the surface of an antigen-presenting cell (APC)—a mechanism restricted to MHC class II cells—or direct stimulation of CD8+ T cells by endogenous antigen—a mechanism restricted to MHC class I cells [14]. Although CMI seems to be more important for effective tumor cell killing, both humoral and CMI mechanisms can be important for optimal immunologic effect.

Active immunotherapy uses immunostimulants and vaccines, whereas passive (adoptive) immunotherapy transfers activated immune components from a sensitized host to the affected individual. One of the passive immunotherapies under evaluation is administration of interleukin-2 (IL-2) stimulated tumor-infiltrating lymphocytes to patients with malignant melanoma [15]. Another approach to passive immunotherapy is the administration of monoclonal antibodies that recognize specific tumor antigens [16,17].

IMMUNOGENIC ANTIGENS

Melanoma antigens can be divided into two broad categories: tumor-associated antigens (TAA) and melanoma-associated antigens (MAA) (Table II) [18]. TAA are common to melanoma cells and other tumor cells. They can be cell-surface products seen in embryologic tissues, proto-oncogene products, or antigens associated with viral transformation [19]. MAA are found predominantly in melanomas, but also are often expressed in normal melanocytes. MAA are usually proteins or glycoproteins, and several have been well defined [20–32].

The selection of TAA and MAA for a melanoma vaccine is extremely problematic, in part because expression of antigens in tumor cells is inherently heterogeneous. Thus a tumor's antigenic profile may change in response

TABLE III. Advantages/Disadvantages of Different Vaccine Preparations

Type of vaccine	Advantages	Disadvantages	Examples
Polyvalent			
Whole melanoma cells (autologous or allogeneic)	Invokes broad range of protective responses	Availability of autologous cells	
	May circumvent TAA[a] heterogeneity	Irrelevant cellular material	Polyvalent melanoma cell vaccine (PMCV)
	More immunogenic	May contain immune suppressive factors	
	Less vulnerable to antigenic modulation	Difficult to characterize and reproduce	
		Theoretically more toxic	
Cell lysate	(same as above)	(same as above)	Vaccinia melanoma cell lysate (VMCL)
Partially purified antigens	Less irrelevant material	Difficult to characterize and reproduce	Shed-antigen vaccine and mechanical cell lysates
Univalent			
Purified antigens	Minimal irrelevant material	Less immunogenic	GM2-KLH/QS-21
Peptide antigens	Theoretically less toxic	Very vulnerable to antigenic modulation	
	Easier to characterize and reproduce	Necessary to match TAA and/or HLA type	

[a]Tumor-associated antigens.

to the host's immune response, or a tumor antigen may encrypt itself so that it is not immunologically recognized. Another problem is that most TAA and MAA are weakly immunogenic. An antigen's immunogenicity can be increased by changing its composition or altering its physical attributes or biochemical properties. The former can be achieved by physical aggregation [33] and the latter by enzymatic treatment [34], or coupling the antigen to immunomodulatory agents such as cytokines [35]. Additionally, as described above, adjuvants are often added to a vaccine as nonspecific immune enhancers. A vaccine's effect also can be modulated by concurrent administration of immunogenic agents [36,37], or inhibitors of immune suppression [38]. Recently, there has been major emphasis on the use of genetic engineering to increase immunogenicity by introducing genes that encode cytokines or cell-surface immune accessory molecules [39].

CURRENT APPROACHES TO ACTIVE IMMUNOTHERAPY OF MELANOMA

The development of technology to identify and characterize melanoma antigens has shifted attention from immunostimulants to antigen-specific immunotherapy with vaccines. Melanoma vaccines range from complex antigen mixtures, such as whole cell preparations, to purified single antigens. Complex vaccines are polyvalent and therefore can stimulate immune responses to many tumor antigens, which increases the strength of the overall immune response. In addition, the antitumor activity of a polyvalent vaccine is less susceptible to antigenic modulation by cancer cells, although immune responses to irrelevant antigens are a potential disadvantage of complex vaccines. Vaccines made from purified antigens or peptides are easier to manufacture and quality control, and the patient's response to a single antigen is easier to study. However, single-antigen vaccines can be defeated by the outgrowth of resistant antigen-negative tumor clones. Hopefully, current clinical trials will resolve the question of which approach is most beneficial (Table III).

Whole-Cell Polyvalent Vaccines

Living whole tumor cells inactivated by irradiation so they are not capable of growth are the most effective immunogens in syngeneic animal tumor host models. Thus, whole cell vaccines have been extensively studied in human trials of active immunotherapy.

Autologous preparations. Because it uses the patient's own tumor as the source of antigen, an autologous vaccine is patient-specific, and its production depends on the availability of tumor cells from that patient. Berd and Mastrangelo [38] have conducted extensive studies of autologous whole-cell melanoma vaccines. Their vaccine is prepared by modifying the methodology of Peters et al. [40]. Freshly excised tumor masses are minced, placed in an enzyme solution to dissociate the melanoma cells, and then cryopreserved in liquid nitrogen. Three days prior to immunotherapy, the patient receives intravenous cyclophosphamide as an adjuvant. On the day of vaccination, the tumor cells are thawed, irradiated, and combined with Tice strain BCG. The autologous vaccine is administered by intradermal injections in the extremities. The cycle is repeated every 28 days. Approximately 25% of the patients develop nausea following cyclophosphamide ad-

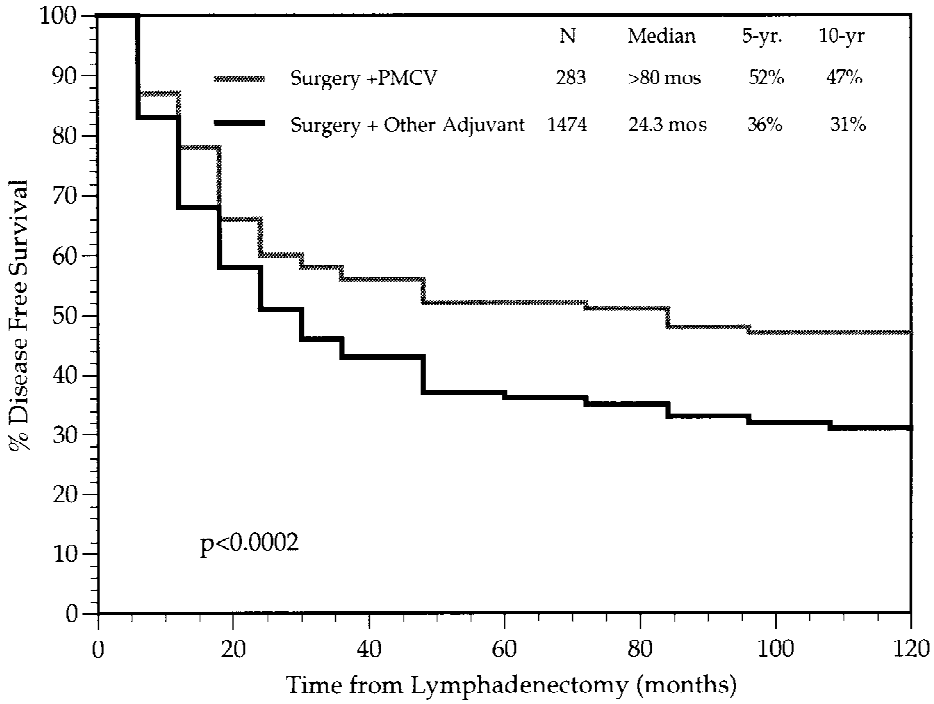

Fig. 1. Disease-free survival of AJCC stage III melanoma patients receiving PMCV vs. other adjuvant therapies after regional lymphadenectomy.

ministration. A local inflammatory response develops at the injection site, but there are no systemic side effects associated with the vaccination.

A nonrandomized trial of autologous vaccine plus BCG demonstrated a 12.5% regression rate [41]. Although the response rate was low, patients who did respond developed statistically significant delayed-type hypersensitivity (DTH) to the vaccine. This suggests that an intact immune response is necessary for vaccine-mediated regression.

Several other approaches have been used in an attempt to increase the immunogenicity of autologous vaccines. Perhaps the most promising is the introduction of cytokine genes such as interleukin 2 or interleukin 4 [42,43].

Allogeneic preparations. The polyvalent ''antigen-enriched'' whole-cell melanoma vaccine (PMCV) at the John Wayne Cancer Institute (JWCI) has evolved over the past three decades. The current vaccine is a live-cell preparation of three allogeneic melanoma cell lines chosen for their high content of immunogenic TAA and MAA [37]. These cell lines are grown separately, harvested, and then combined in equal parts. The vaccine is aliquoted, cryopreserved in liquid nitrogen, and irradiated. During a 12-week induction phase, the patient receives biweekly intradermal PMCV vaccinations, the first two of which are given with BCG. The patient then receives monthly vaccinations for the first year and every 2–3 months thereafter.

The toxicity associated with PMCV is negligible. A minority of patients report some mild fatigue, musculoskeletal discomfort, and hyperpyrexia associated with the first two vaccinations and BCG. A few patients develop an intense local inflammatory skin reaction to BCG. This is self-limiting, but occasionally it requires local wound care.

Phase II trials of PMCV for AJCC stage IV melanoma reported a significantly higher median survival for 157 vaccine recipients than for 1,521 historical control patients receiving other therapies (23 vs. 7.5 months, respectively; $P = 0.0001$) [44]. The survival benefit was greatest in patients rendered free of disease by surgical resection before vaccine therapy: JWCI's 5-year survival rate of 10% following complete resection of distant melanoma metastases increased to 33% when patients received PMCV postoperatively [18].

Phase II trials of PMCV for AJCC stage III melanoma revealed a significantly higher median survival for 283 patients receiving adjuvant therapy with PMCV following complete resection of regional metastases than for historical controls receiving other postoperative adjuvant therapies (>80 vs. 24 months, respectively) (Fig. 1). The two groups had similar prognostic factors, including a similar distribution of tumor-involved lymph nodes (Fig. 2). Five-year and 10-year rates of overall survival were 52% and 47%, respectively, in patients receiving PMCV, vs. 36% and 31%, respectively, for historical controls. A phase III prospective randomized trial of adjuvant PMCV vs. interferon alfa-2b for patients with resected AJCC stage III melanoma is scheduled to begin this year.

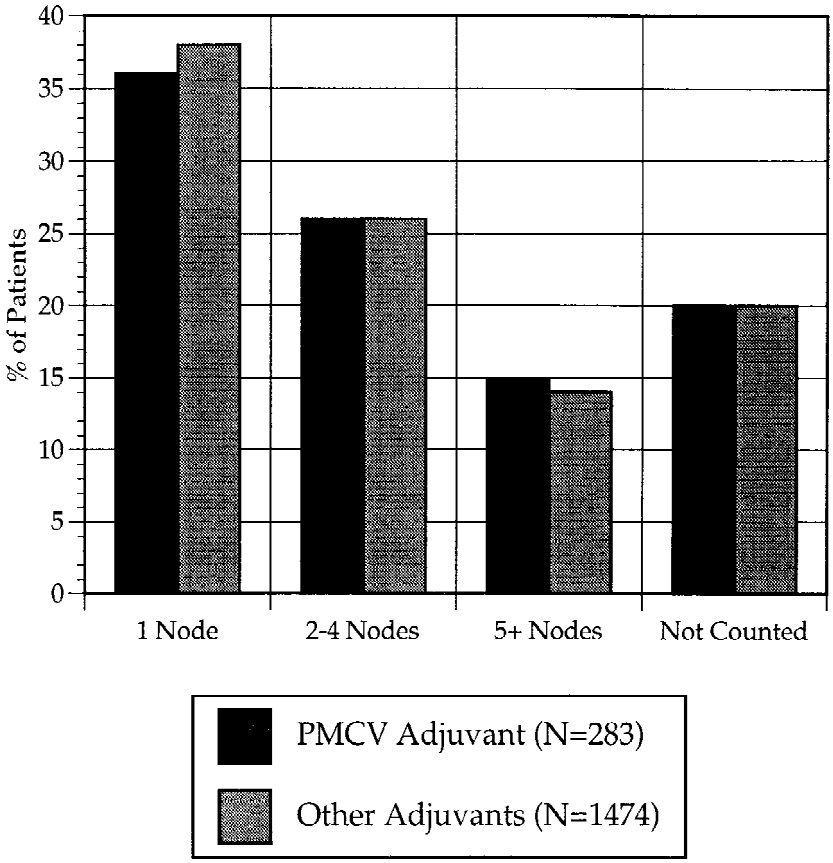

Fig. 2. Number of tumor-involved lymph nodes in AJCC stage III melanoma patients receiving PMCV vs. other adjuvant therapies after regional lymphadenectomy.

Lysate Vaccines

Viral lysates. The ability of a virus to induce long-lasting antitumor immunity via viral-induced oncolysis was first demonstrated in an animal model [45]. Human studies with lysates induced by a wide variety of viruses, including vaccinia [46], suggested a possible therapeutic role for viral lysates. The vaccinia melanoma oncolysate (VMO) vaccine developed by Wallack [46] was made by infecting four established allogeneic melanoma cell lines with live vaccinia virus. Each melanoma cell line was infected separately and incubated overnight. The infected cells were separated by centrifugation and a nucleus-free cell lysate extracted. The lysates of the four cell lines were then pooled in equal cellular concentrations to produce a tetravalent VMO vaccine.

One week before beginning VMO immunotherapy, each patient received a vaccinia booster vaccination. The vaccine was injected intradermally over the regional lymphatic basins weekly for 13 weeks and then biweekly for 1 year. Toxicity was minimal; headaches, nausea, and low-grade fevers were the most common complaints.

The statistically significant prolongation in disease-free interval demonstrated in a phase II trial of postoperative VMO adjuvant immunotherapy [47] was not confirmed in a phase III prospective, randomized multi-institutional trial of VMO vaccine vs. vaccinia virus alone [48]. Subset analysis suggested that males may derive a benefit, but the trial was not large enough to answer this question definitively.

Hersey [49] has developed a vaccinia melanoma cell lysate (VMCL) vaccine using methodology similar to that used by Wallack [46], but with only one allogeneic cell line. VMCL is administered intradermally over the deltoid or anteromedial thigh, rotating to a different site with each injection. Vaccine induction is 6 months of injections every other week or every 3 weeks, with cyclophosphamide as an adjuvant. The maintenance schedule is once a month. A phase II trial of VMCL for AJCC stage III melanoma demonstrated 5-year survival rates of 50% for vaccine recipients vs. 34% for historical controls [50]. A prospective, randomized study of VMCL versus no immunotherapy is nearing completion.

Mechanical lysates. Mitchell et al. [51] used a high-speed tissue homogenizer and three freeze-thaw cycles to create a vaccine from lysates of two melanoma cell lines. This lysate vaccine (Melacine) is administered with the adjuvant DETOX (monophosphoryl lipid A and a purified mycobacterial cell-wall skeleton), after administra-

tion of intravenous cyclophosphamide (300 mg/m^2) as an additional adjuvant. Melacine injected subcutaneously into the upper extremity and buttocks on weeks 1, 2, 3, 4, and 6 is associated with minimal soreness at the injection site and no systemic toxicity beyond flu-like symptoms [52,53]. Interim results from a multicenter phase II trial showed rates of 3% for complete response, 5% for partial response, and 27% for minor response or stable disease among 139 AJCC stage III and IV melanoma patients [54]. A recent randomized phase III trial of Melacine vs. combination chemotherapy for AJCC stage IV melanoma revealed no difference in median survival. Eighteen patients who failed Melacine were given interferon alfa-2b at a dosage of 5×10^6 U/m^2 subcutaneously, three times per week: eight demonstrated a partial response [55]. Underway is a phase III trial comparing Melacine plus interferon alfa-2b with interferon alfa-2b alone[56].

Shed Antigen Vaccines

Bystryn [61] has developed a partially purified, polyvalent melanoma antigen vaccine made from surface material shed into a culture medium by a pool of selected melanoma cells. This shed-antigen vaccine theoretically provides a broad array of ganglioside and other tumor antigens without the irrelevant and perhaps immunosuppressive factors that may be present in whole-cell or even cell lysate preparations. The shed-antigen vaccine is prepared from melanoma cell lines selected for their differential expression of surface TAA. Cells are grown in serum-free media and the shed material is harvested, characterized, and purified. The shed-antigen vaccine elicited humoral and/or cellular immune responses that were correlated with improved survival. Alum seems to be the most effective adjuvant with this shed-antigen preparation.

Carbohydrate Antigen Vaccines

The early work of Livingston and associates [57,58] with vaccines containing purified ganglioside antigens, namely, GM_2, was initially very exciting because of the IgM antibodies produced. These antibodies lysed melanoma cells expressing the GM_2 antigen via a complement-mediated pathway. However, no cytotoxic T cells were induced and no IgG antibodies were produced. The GM_2 antigen was obtained from brain tissue of cats with Tay-Sachs disease through a series of separations and purifications. GM_2 was also obtained from bovine brain tissue by cleaving a terminal galactose on the GM_1 ganglioside. Purified GM_2 was then placed in suspension with Tice strain BCG, thereby creating the vaccine.

One week prior to the first and fourth vaccinations, cyclophosphamide was administered intravenously as an adjuvant. The vaccine was administered via intradermal injections (6–10/vaccination) into an extremity biweekly for three sessions; then maintenance immunizations were given at 2 and 5 months. Cyclophosphamide caused some mild, self-limiting nausea, and BCG produced an intense, local skin response at the site of injection. There was no toxicity associated with the GM_2 moiety of the vaccine.

A prospective, randomized trial compared GM_2/BCG vaccine with BCG alone after complete surgical resection of AJCC stage III melanoma [59]. GM_2/BCG immunotherapy induced an IgM antibody response in a majority of patients but did not improve disease-free or overall survival. However, subset analysis revealed that patients who produced the GM_2IgM antibody did have a longer disease-free and overall survival.

To improve the humoral response, GM_2 has been conjugated to keyhole limpet hemocyanin (KLH), which acts as the carrier protein, and administered with the adjuvant QS-21. This GM_2-KLH/QS-21 vaccine is superior to the GM_2/BCG vaccine with respect to generation of cytotoxic antibodies [60]. Underway is a phase III trial of GM_2-KLH/QS-21 vs. interferon alfa-2b for patients who have undergone surgical resection of AJCC stage III melanoma.

RECENT ADVANCES AND FUTURE DIRECTIONS

Although randomized trials have yet to show a significant improvement in the survival of patients receiving melanoma vaccine therapy, recent advances in tumor immunology suggest new approaches that may improve results. The humoral and cellular response to melanoma vaccine immunotherapy can be maximized by identification of the most immunologically relevant tumor antigens, more efficient presentation of these antigens, and more potent immunomodulation using costimulatory molecules and cytokines. Advances in our understanding of adjuvants, vaccine dosing and administration, and monitoring the response to immunotherapy should also increase the efficacy of melanoma vaccine therapy.

Antigen Selection

The availability of recombinant antigen allows the characterization of antigen-specific humoral and cellular responses in patients receiving vaccine therapy. Correlation of these responses with clinical outcome should allow identification of the most immunologically relevant antigens. Efforts can then focus on augmenting clinically favorable responses by presenting the appropriate antigens as components of a cellular, protein, or peptide vaccine. Induction of specific CTL, DTH, and antibody responses to tyrosinase, MAGE-1, and other melanoma-associated peptides in patients treated with PMCV has recently been demonstrated [31,49,62–64].

Several protein or peptide MAA are being utilized as vaccines in phase I clinical trials, with or without adjuvants. Results of these trials will determine the relative

immunogenicity and clinical efficacy of each antigen as an immunotherapy target and facilitate the design of an optimal multivalent vaccine. Evaluation of the expression of these or other TAA by a patient's tumor may ultimately allow tailoring of a vaccine to match the antigenic profile of the patient's tumor. Perhaps more importantly, the same research approaches that led to the identification and cloning of existing TAA are likely to identify a series of new antigens in the future. This will expand the number of potential targets and increase the chance of developing a more effective immunotherapy.

The importance of gangliosides as immunogenic antigens in melanoma patients is now being recognized [57–59]. The gangliosides GM_2, GD_2, GM_3, GD_3, and O-acetyl-GD_3 are present on the surface of many melanoma cells and are immunogenic in patients with melanoma [57,65–69]. Of these, GM_2 is the most consistently expressed and immunogenic antigen and has received the greatest interest as a potential immunotherapy target [57–59]. GM_2 is a glycolipid and therefore is not HLA-restricted and generates only an antibody response, primarily of the IgM isotype. The IgM response to the GM_2/BCG vaccine has been correlated with survival [59], and the current phase III trial of the GM_2/KLH conjugate vaccine will probably reveal a similar but perhaps stronger correlation. At JWCI, an enhanced IgM response to the gangliosides GM_2, GD_2, GM_3, and GD_3 has been correlated with survival in patients receiving PMCV [68] (Hoon DSB and Irie RF, unpub. data). These data suggest an important role for gangliosides as components of multivalent cellular or molecular vaccines.

Other cell-surface glycoproteins that form the tumor glycocalyx also may be important targets for HLA-unrestricted humoral immune responses in melanoma patients. Ravindranath and colleagues [70] recently identified high levels of expression of the Lewis blood group antigens sialyl-Lewis^x (sLe^x) and sialyl-Lewis^a (sLe^a) on the surface of melanoma cells, including those of PMCV. This high level of expression was not evident in normal melanocytes, and an enhanced antibody response to these antigens was seen in patients treated with PMCV. A possible correlation between anti-sLe antigen response and clinical outcome is being investigated.

Phenotype of Immune Response

The many different types of immune response are highly unlikely to have the same clinical antitumor effect. As discussed above, immune responses can be classified as humoral (antibody) or cellular. Humoral immune responses can be predominantly IgM, IgG, or other immunoglobulin classes and can vary in affinity for antigen. Cellular responses can be mediated by CD4+ T lymphocytes (which mediate classic DTH) or cytotoxic CD8+ T cells. The type of antitumor immune response may be as important as its presence.

Several studies have examined the clinical correlates of different immune responses to melanoma vaccine. For example, Jones et al. [71] studied the immune response to the glycoprotein antigen TA-90 in patients receiving PMCV. A strong IgM and DTH response to this antigen correlated favorably with survival, whereas a strong IgG response correlated inversely with survival. These data formed the basis of an immunologic survival model for patients treated with PMCV. If this or similar models can be validated in a large cohort of patients, more objective assessment of the immunologic response of a particular patient to immunotherapy will be possible. Patients developing a favorable response could then continue on the same immunotherapy, whereas those failing to do so could be switched to another form of therapy. This should maximize clinical efficacy by individualizing therapy based upon responsiveness.

Antigen Presentation

Antigen presentation either by specialized APC or by tumor cells themselves is required for development of an effective antitumor CTL response. Because a patient's tumor has multiple potentially immunogenic tumor antigens, an ineffective immune response could be considered a failure of antigen presentation [72]. Several strategies to increase the efficiency of tumor antigen presentation during vaccine therapy have therefore been developed. Cytokines such as interferon-alfa, interleukin-2 (IL-2), interleukin-4 (IL-4), and GM-CSF can upregulate antigen expression on melanoma cells, enhance helper T-cell function, and augment antigen presentation [73]. However, systemic administration of these agents produces significant toxicity [74]. A cytokine-transduced cellular vaccine (allogeneic or autologous), or administration of liposomal or microencapsulated cytokine preparations with vaccine [75] avoids systemic toxicity and delivers high concentrations of cytokines at the vaccine site where antigen presentation will initially occur. Preclinical studies demonstrate enhanced CMI when whole-cell vaccines are transduced with IL-2, IL-4, IL-7, GM-CSF, or combinations thereof [76–80]. Several phase I and phase II clinical trials evaluating cytokine-transduced cellular vaccines are underway [80–83].

Another strategy to improve antigen presentation is administration of APC with the vaccine [84]. Fibroblasts can function as direct APC, and injection of autologous cytokine-secreting fibroblasts with vaccine augments CMI to TAA in animal models [85]. Underway is a clinical trial of autologous melanoma cell vaccine administered with cytokine-transduced autologous fibroblasts (M. Lotze, Univ. Pittsburgh). It is also possible to isolate and culture autologous APC derived from peripheral blood or bone marrow [86,87]. These cells can be pulsed with peptide tumor antigens in vitro and then administered as a vaccine. Induction of antigen-specific CMI by

peptide-pulsed APC has been demonstrated in animal models [87–89], and clinical studies have shown similar immune responses after peptide-pulsed APC vaccine therapy [90,91].

The CTL response to tumor antigens also can be improved by upregulating costimulatory and adhesion molecules on vaccine cells. CTL recognition of a peptide antigen requires presentation of the antigen in the context of the appropriate class I MHC molecule as well as costimulatory and adhesion molecules such as B7, LFA-3, and ICAM-1. Since many tumor and vaccine cells do not express high levels of these molecules, augmenting their expression can increase the CTL response to certain antigens. This has been demonstrated in animal models for both class I MHC and B7 [92,93] and formed the theoretical basis for a current trial of a B7-transduced, class I MHC-matched allogeneic melanoma cell vaccine (M. Sznol, NIH).

Vaccine Dosage and Administration

Perhaps the least studied aspects of vaccine therapy are dosage and administration. Pharmacokinetics and drug dose are easily measured for standard drug treatments but difficult to measure for immunotherapy. Until recently, it was impossible to quantitate the dose of antigen because all vaccines were whole cells or cell lysates containing an unknown quantity of antigen. With the advent of molecular (protein, peptide, or ganglioside) vaccines, it is now possible to quantify antigen dose. Although preclinical data indicate that there is a minimal dose of antigen required to elicit a measurable response, the exact dose required appears to vary from antigen to antigen and from host to host. There also appears to be a maximal antigen dose for certain antigens, above which the immune response is inhibited. Unfortunately, there are no data on the most effective doses for specific antigens. Similarly, there are few clinical data directly comparing different immunization schedules for specific tumor antigens. Previous studies evaluating the immune response to whole-cell vaccine indicate that weekly or biweekly vaccinations are most effective during the early (induction) phase, but the optimal frequency and duration of maintenance therapy is less clear (Morton DL, unpub. obs.). Ongoing studies involving molecular vaccines should help answer these important questions.

CONCLUSIONS

Immunotherapy for melanoma has reached an exciting phase in its evolution. Although a randomized clinical trial has yet to show a statistically significant improvement in the survival of patients receiving vaccine therapy, several studies have shown enhanced survival of patients developing an immune response to the vaccine. The knowledge and techniques of modern molecular biology and immunology suggest multiple strategies to augment the host response to a melanoma vaccine. The challenge of immunotherapy research is to determine which combination of approaches leads to a favorable clinical response and how to monitor that response effectively. The knowledge and experience gained in designing an effective melanoma vaccine may then be applied to developing effective immunotherapies for other malignancies.

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